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Citation for published version:

Glass, EJ, Stewart, J & Weir, DM 1986, 'Altered immune function in alloxan-induced diabetes in mice', *Clinical & Experimental Immunology*, vol. 65, no. 3, pp. 614-21.

Link:

[Link to publication record in Edinburgh Research Explorer](#)

Document Version:

Publisher's PDF, also known as Version of record

Published In:

Clinical & Experimental Immunology

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Altered immune function in alloxan-induced diabetes in mice

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(Accepted for publication 7 May 1986)

SUMMARY

Macrophage lectin receptors that recognize bacterial cell wall sugars are reduced in alloxan diabetic mice. This is in contrast to the expression of macrophage Fc (IgG_{2b}) receptors which remains unaltered. Peritoneal macrophages, from diabetic and normal mice, were used as a source of accessory cells in an antigen dependent T cell proliferation assay with unopsonized *Staph. epidermidis* as the antigen. Uptake of this antigen in the absence of serum is via the macrophage lectin receptors. We have shown that diabetic macrophages induce a level of antigen dependent T cell proliferation to *Staph. epidermidis*. However the T cell response to Con A was similar with both normal and diabetic macrophages. We suggest that the observed defect in antigen presentation by diabetic macrophages is at the level of uptake of antigen. High glucose levels, such as those found in diabetes, down-regulate the lectin receptor, reduce phagocytosis of *Staph. epidermidis* and affect antigen presentation. This has important consequences in terms of the ability of diabetics to mount an effective immune response.

Keywords macrophage lectin receptor diabetes antigen presentation

INTRODUCTION

Immune function in diabetes is disturbed (Ganda, 1983; Pasko, Salvin & Winklestein, 1981; Fletcher-McGruder & Gerritsen, 1984) and this has effects both in the pathogenesis of the disease and in resistance to infection. However the site(s) and mechanism(s) for the observed increased susceptibility to infection in diabetes are not clear.

Macrophages are involved in the initiation of humoral and cell mediated immune responses and also have effector functions, including the removal of immune complexes and infective agents and the release of numerous factors. They depend for their activity on a variety of receptor molecules present in their plasma membrane that act as recognition sites. These range from immunoglobulin and complement receptors to products of the major histocompatibility complex (MHC). One of these surface molecules which we have described is a lectin-like receptor on phagocytes that binds unopsonized bacteria by their cell wall sugars (Glass, Stewart & Weir, 1981; Freimer *et al.*, 1978). Changes in the expression of any of these surface structures is likely to have important consequences for the normal functioning of the cells of the immune system.

In previous reports we have shown that a variety of environmental influences affect receptor expression (Weir, Blackwell & McLean, 1981; Glass, Stewart & Weir, 1983). These studies indicated that D-glucose blocks the macrophage lectin receptor at concentrations likely to be present in diabetes (10–20 mM). We have also shown that diabetic mice became highly susceptible to infection by a *Salmonella typhimurium* mutant that has glucose as its terminal LPS sugar. Thus it would

appear that increased blood glucose levels in diabetes down-regulate the macrophage lectin receptor. Other workers (Summerfield, Vergalla & Jones, 1982; Pizzo *et al.* 1981) have also shown a reduction in lectin receptors that recognize asialoglycoproteins in diabetes. In the present report these findings have been extended both with respect to the expression of macrophage membrane receptors in experimental diabetes and to their function in the initiation of an immune response using a lymphocyte proliferation assay.

MATERIALS AND METHODS

Animals. C3HfBu/Kam male mice (aged 6–12 weeks) were bred in the department in a conventional mouse colony.

Diabetic mice. These were injected intravenously with 0.5 ml alloxan (3 mg/ml; BDH, Poole, Dorset) following 6 h starvation. Urine was tested for the presence of glucose using Diastix. In some experiments plasma glucose was also determined using a glucometer and Dextrostix (Ames Division, Mile Laboratory Ltd, Slough, UK) (glucose oxidase test).

Preparation of cells. Resident peritoneal macrophages were obtained by peritoneal lavage with 10 ml Hanks' balanced salt solution. Inguinal and periaortic lymph node cells were obtained from mice injected subcutaneously in the tail base with 0.1 ml of an equal volume of formalin-killed *Staphylococcus epidermidis* at 6×10^9 organisms/ml and Freund's complete adjuvant, 10–11 days previously. T lymphocytes were separated on nylon-wool columns and resuspended in RPMI 1640 (Julius, Simpson & Herzenberg, 1973).

Preparation of *Staph. epidermidis*. A strain of *Staph. epidermidis* was obtained from the department teaching collection. The bacteria were grown in nutrient broth (Oxoid Ltd, Wade Road, Basingstoke, UK) harvested in log phase and killed by 24 h exposure to 0.5% formalin at 4°C.

Preparation of EA. EA_G was prepared essentially as described elsewhere (Glass, Stewart & Weir, 1982) except that the anti-sheep erythrocyte antibody used as mouse monoclonal IgG2b (MAS 013c, Clone Sp2; Sera Lab Ltd, Crawley Down, Sussex, UK).

Preparation of monolayers. Macrophage monolayers were prepared as previously described (Glass *et al.*, 1982).

Bacterial binding assay. The macrophage monolayers were overlaid with 1 ml of bacteria in Dulbecco's PBS (D.PBS) containing Ca²⁺ and Mg²⁺ ions (0.9 mM and 0.4 mM respectively) and incubated for 2 h at 4°C. Non-attached organisms were removed by repeated washing with D.PBS.

Fc rosettes. Two millilitres of EA_G (2.5×10^7 erythrocytes/ml) were added to each monolayer, centrifuged for 5 min at 100 *g* and incubated for 40 min at 37°C. Non-adherent red cells were removed by very gentle washing with Hanks' balanced salt solution.

Counting method. Red cell and bacterial binding were estimated microscopically by counting macrophages with red cells or bacteria attached at two or more discrete points. Two hundred macrophages were counted per monolayer and the results were expressed as the percentage of cells binding erythrocytes or organisms.

Proliferation assay. Resident peritoneal cells (100 μ l; 1×10^6 /ml) in RPMI 1640 without serum were incubated in 96-well flat bottomed tissue culture plates for 1 h at 37°C. Non-adherent cells were removed by washing with RPMI 1640 without serum. After treatment with 2.5 μ g mitomycin C per well for 20 min at 37°C the macrophage monolayer were cultured for 5 h with various dilutions of *Staph. epidermidis* in RPMI 1640 without serum, or medium alone. The monolayers were washed and either 2×10^5 nylon-wool enriched T cells in RPMI 1640 + 5% fetal calf serum for antigen dependent proliferation, or 2×10^5 nylon-wool enriched T cells in RPMI 1640 + 5% fetal calf serum containing various amounts of Con A, for mitogen induced proliferation, were added. The cultures were incubated at 37°C in a humidified CO₂ incubator for 3 or 5 days for mitogen-induced and antigen-dependent proliferation respectively. The cultures were labelled for the final 24 h with 1 μ Ci tritiated thymidine (Amersham International plc, Amersham, Bucks, UK) per well. In all experiments quadruplicate cultures were prepared.

Immunoperoxidase measurement of Mac-1 and Ia. Macrophage monolayers were prepared as described above. Cell suspension of total peritoneal cells were set up in minisorb tubes (Nunc,

Denmark) (2×10^6 cells per tube). These two cell preparations were incubated with latex beads (Difco Laboratories, West Molesey, Surrey) for 1 h at 37°C. Non-phagocytosed latex was removed from adherent cell monolayers by vigorous washing. The total peritoneal cell suspensions were washed three times by centrifugation and smears prepared on glass slides. All the following procedures were carried out at room temperature. Cells were fixed for 20 min with 1% paraformaldehyde and endogenous peroxidase activity blocked by 0.3% hydrogen peroxide in methanol for 30 min. The cells were then washed with 2% normal rabbit serum (NRS) (SAPU Law Hospital, Carlisle, Lanarkshire) in 5 mM Tris buffered saline (TBS), pH 7.6, and Fc receptors blocked with 20% NRS in TBS for 10 min. Specific antibody was added—either Mac-1 (neat supernatant fraction from American Type Culture Collection, Rockville, Maryland, No TIB 128) or 1/50 dilutions of A.TH anti A.TL (Sera Lab, Sussex, UK) which was the lowest concentration that gave the highest number of detectable positive cells. After incubating for 30 min the cells were washed with 2% NRS in TBS and horseradish peroxidase conjugated anti-mouse immunoglobulin (P161, Dakopatts, Denmark) for Ia detection, or anti-rat immunoglobulin (P162 Dakopatts, Denmark) for the detection of Mac-1 was added for 30 min. The cells were thoroughly washed with TBS and aminoethylcarbazole (Sigma, Poole, Dorset, UK) (0.4% in dimethylformamide, diluted 1:10 before use with 0.1 M sodium acetate buffer and containing 0.01% hydrogen peroxide) was added for 5 min. The cell preparations were washed and counterstained with haematoxylin for 45 s, washed and mounted in glycerine jelly. Background staining in the absence of Mac-1 or A.TH anti A.TL was negligible and was subtracted from the results obtained with the antibodies.

Statistical analysis. Results were compared using the non-paired Student's *t*-test.

RESULTS

Receptors and macrophage antigens. Blood glucose levels in mice injected with alloxan or normal control mice were determined (Table 1). By day 3 blood glucose concentration had risen from a normal value of 8.1 ± 0.5 to 21.5 ± 3.0 mmol/glucose. A peak glucose level was reached on day 5 that represented a 4-fold increase from day 0 and thereafter remained at this high level. Concurrently *Staph. epidermidis* binding to peritoneal macrophages in the absence of serum (lectin receptor binding) and Fc receptor expression were measured (Fig. 1 and Table 2 respectively). Lectin receptor expression decreased significantly at all doses of *Staph. epidermidis* except 10^7 organisms/ml after alloxan treatment. By day 3 the percentage of macrophages binding *Staph. epidermidis* at the three higher doses was reduced considerably ($P < 0.05$). Seven days after alloxan treatment the level of binding was decreased by 50% at the highest dose of organisms ($P < 0.005$). The diminished expression of lectin receptors binding *Staph. epidermidis* was maintained throughout the duration of the experiment (15 days). In contrast Fc (IgG2b) receptors decreased

Table 1. Blood glucose levels after alloxan injection

Day after alloxan injection	Blood glucose level (mmol/l \pm s.e.m.)
0	8 ± 0.5
1	6 ± 3
3	21 ± 3
5	32 ± 4
7	26 ± 4
10	28 ± 2
15	22 ± 2

Each result represents the mean of five to eleven experiments \pm 1 s.e.m.

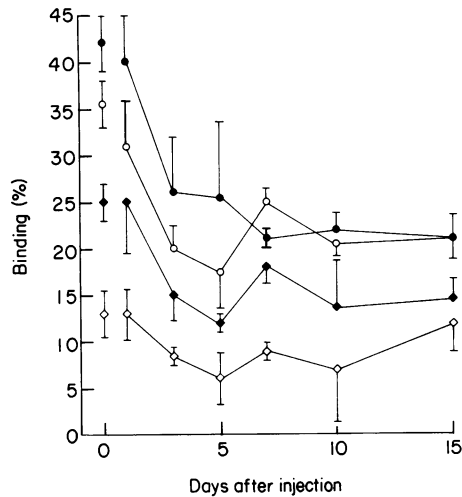


Fig. 1. Binding of *Staph. epidermidis* to mouse peritoneal macrophages after alloxan injection. *Staphylococcus epidermidis* concentrations: 3.3×10^8 organisms/ml (●); 10^8 organisms/ml (○); 3.3×10^7 organisms/ml (◆); 10^7 organisms/ml (◇). Each point represents the mean of four experiments ± 1 s.e.m.

Table 2. Fc receptor expression on peritoneal macrophages after alloxan injection

Dilution of antibody $\times 10^3$	Rosettes (% ± 1 s.e.m.) Day after alloxan injection						
	0	1	3	5	7	10	15
3.1	14 \pm 3	13 \pm 2	7 \pm 2	6 \pm 2	7 \pm 2	10 \pm 4	10 \pm 2
6.2	37 \pm 6	35 \pm 13	21 \pm 2	27 \pm 7	27 \pm 10	43 \pm 8	35 \pm 6
12.5	55 \pm 3	45 \pm 8	45 \pm 8	50 \pm 12	37 \pm 9	64 \pm 7	54 \pm 6
25	71 \pm 2	64 \pm 5	59 \pm 3	64 \pm 7	48 \pm 7	73 \pm 9	70 \pm 3
100	84 \pm 2	78 \pm 3	76 \pm 3	74 \pm 5	78 \pm 3	80 \pm 6	80 \pm 5

Each result represents the mean of three to four experiments ± 1 s.e.m.

slightly but not significantly (Table 2) and by day 10 had returned to their original level. Mac-1 and Ia expression were also determined (Table 3) on total peritoneal and adherent peritoneal cells from diabetic and control mice. No differences in the numbers and types of cells obtained from normal or diabetic mice were observed (data not shown). In addition no significant difference in the uptake of latex was detected. Cells capable of phagocytosing latex particles (latex positive) accounted for $59 \pm 6\%$ of normal total peritoneal cells and $49 \pm 9\%$ of diabetic cells. As expected, a greater proportion of the adherent cells were latex positive and Mac-1 positive. No differences were observed in Ia or Mac-1 antigen expression in total peritoneal or adherent cell population after alloxan treatment.

Antigen dependent lymphocyte proliferation. The ability of macrophages from diabetic mice to induce antigen dependent T cell proliferation was compared with normal macrophages. Peritoneal macrophages from mice that had been injected with alloxan 7 days previously showed a maximum reduction in *Staph. epidermidis* binding (see Fig. 1). These macrophages also showed diminished phagocytosis of unopsonized *Staph. epidermidis* (at 10^8 /ml *Staph. epidermidis* there was a 52.3% reduction in the percentage of macrophages that phagocytosed the organism). At all concentrations

Table 3. Comparison of Ia antigen and MAC-1 antigen expression, and latex uptake on peritoneal cells (p.c.) from normal or diabetic mice

Phenotype	Cells (% positive \pm 1 s.e.m.)			
	Total p.c. normal	Total p.c. diabetic	Adherent p.c. normal	Adherent p.c. diabetic
Latex +ve/mac-1 +ve	38 \pm 4	34 \pm 7	72 \pm 3	77 \pm 4
Latex -ve/ma-1 -ve	36 \pm 6	47 \pm 10	6 \pm 1	4 \pm 1
Latex +ve/mac-1 -ve	22 \pm 4	15 \pm 4	16 \pm 3	13 \pm 3
Latex -ve/mac-1 +ve	4 \pm 1	5 \pm 2	6 \pm 1	8 \pm 1
Latex +ve/Ia +ve	10 \pm 1	9 \pm 0.5	15 \pm 1	15 \pm 2
Latex -ve/Ia -ve	4 \pm 1	4 \pm 0.3	2 \pm 0.6	3 \pm 0.4
Latex +ve/Ia -ve	68 \pm 2	69 \pm 1	82 \pm 1.5	85 \pm 2
Latex -ve/Ia +ve	18 \pm 0.5	18 \pm 0.1	0.6 \pm 0.2	0.5 \pm 0.2

Each result represents the mean percentage of cells that possessed each phenotype \pm 1 s.e.m. from three to eight experiments.

Table 4. The stimulation index

$$\left(\frac{\text{mean ct/min incorporated in presence of antigen}}{\text{mean ct/min incorporated without antigen}} \right)$$

from five separate experiments comparing peritoneal macrophages from normal and alloxan-induced diabetic mice as accessory cells

<i>Staph. epidermidis</i> concentration (cells/ml)	Source of macrophages	Stimulation index				
		Expt 1	Expt 2	Expt 3	Expt 4	Expt 5
3.3×10^6	Normal	5.82	8.97	5.91	17.5	9.2
	Diabetic	4.46	2.27	4.48	6.74	6.8
10^6	Normal	4.54	5.25	3.39	12.53	7.4
	Diabetic	1.93	1.21	2.29	4.93	4.9
3.3×10^5	Normal	2.84	2.71	1.69	10.78	5.1
	Diabetic	1.05	1.11	1.33	4.7	3.2
10^5	Normal	2.84	1.28	1.31	6.65	2.8
	Diabetic	1.18	1.0	1.33	2.51	2.3

The mean ct/min ^3H -thymidine in the control culture (without antigen) was 1064 ± 321 with normal macrophages and 1221 ± 341 with macrophages from diabetic mice.

of *Staph. epidermidis* the macrophages from normal mice induced a greater level of antigen dependent T-cell proliferation than those from diabetic mice (Table 4). There was no difference in the control values for normal and diabetic macrophages without antigen and no proliferation was observed in the presence of an unrelated antigen (sheep red blood cells). T cells in the absence of adherent cells did not proliferate to *Staph. epidermidis* (data not shown). The absolute counts per

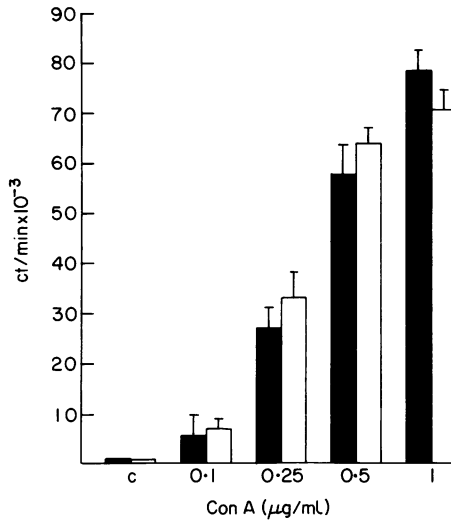


Fig. 2. Incorporation of ^3H -thymidine by T cells responding to Con A in the presence of normal macrophages (■) or diabetic macrophages (□). Each bar represents the mean of five experiments ± 1 s.e.m.

minute of incorporated ^3H -thymidine varied considerably between experiments, although the overall pattern was always the same, so the results in Table 4 are expressed as stimulation indices. In contrast the macrophage dependent T cell proliferation to Con A showed no significant differences (Fig. 2). In fact at 0.25 and 0.5 $\mu\text{g/ml}$ Con A, the diabetic macrophages induced slightly greater proliferation.

DISCUSSION

The results of this study support our earlier findings that suggested that phagocyte activity is altered in mice with alloxan-induced diabetes (Weir *et al.*, 1981). The observation that there is reduced binding and phagocytosis of *Staph. epidermidis* by the peritoneal macrophages together with a reduced ability of the macrophages to induce antigen dependent lymphocyte proliferation to *Staph. epidermidis* is consistent with an impaired ability of the mice to deal effectively with microbial infections and supports our earlier finding on susceptibility to *Salmonella typhimurium* infection.

The mechanisms for the down-regulation of the lectin receptor and reduced antigen-dependent response with diabetic macrophages are not clear. There appears to be a specific effect on the lectin receptor in that Fc receptor expression remains unchanged and phagocytosis of latex particles is also normal. The maintenance of these functions indicates that neither alloxan nor the diabetic environment are having a toxic effect on the cells. In addition, alloxan does not appear to directly affect the lectin receptor expression since human Type 1 diabetics also show depressed bacterial binding (Glass *et al.* unpublished) and over short periods of time, administration of insulin can partially restore normal receptor activity (unpublished observation). It would seem unlikely that the depression of lectin receptor expression is simply a result of blocking of the receptor by the elevated blood glucose levels. If this were the case it might be expected to be reversible and the washing procedures and prolonged incubations in the absence of high glucose concentrations would remove the glucose from the receptor.

Modulation of receptor expression by environmental factors appears to be a general phenomenon. Various workers have reported that stimulated and activated macrophages also have

reduced expression of lectin receptors (Glass *et al.*, 1983; Imber *et al.*, 1982; Ezekowitz *et al.*, 1981). Weil & Pizzo (1983) have shown that incubation of macrophage with 25 mM D-glucose for 3 h *in vitro* reduced the number but not the affinity of the lectin receptors that recognize mannose or N-acetyl glucosamine terminated glycoproteins. Earlier work (Pizzo *et al.*, 1981) had also indicated impairment of expression of this lectin receptor in diabetes. Thus high levels of glucose down-regulate lectin receptors possibly by increasing the rate of internalization or degradation or by decreasing the rate of synthesis. It is possible that high concentrations alter macrophage receptor expression indirectly by affecting lymphokine production. However, high glucose concentrations *in vitro* (25 mM or greater), in the absence of any lymphokines, results in down regulation of the mannose/N-acetylglucosamine receptor (Weil & Pizzo, 1983). Thus various stimuli may affect the expression of the receptors.

In contrast to the depressed antigen dependent T cell proliferation, with diabetic macrophages the Con A response is unaltered. Since the T cell response to Con A is macrophage dependent (Ahmann, Sachs & Hodes, 1978), this further indicates that the alloxan treatment is not having a general adverse effect on macrophage function. Diabetic macrophages do support T cell proliferation to a mitogen but not to *Staph. epidermidis*. This suggests that the defect in the diabetic macrophages is at the level of uptake, processing or presentation of the staphylococcal antigens to T helper cells, rather than a defect in other functions such as the production of IL-1. Other workers have found that diabetic macrophages produce suppressor factors (probably prostaglandins) that inhibit the production of IL-2 and thereby reduce the level of T cell proliferation (Prud'homme *et al.*, 1984). However this would not appear to be the case here since the Con A response is normal and supernatant fractions from diabetic macrophages had no effects on T cell proliferation (unpublished observation).

Both attachment and phagocytosis of unopsonized *Staph. epidermidis* occurs via the lectin receptor. Thus down-regulation of the receptor by increased blood glucose levels may lead to defective presentation of any microorganism that is taken up by this mechanism. Other workers have found that phagocytosis of opportunistic microorganisms such as *Candida albicans* and Type II group B streptococci (Edwards & Fuselier, 1983) is reduced in diabetes. Both these organisms are likely to be recognized by the lectin receptor. Diabetes is associated with an increased susceptibility to infection by opportunistic microorganisms and this report suggests a possible mechanism for these observations. Non-impairment of latex uptake suggests that antigens that are not taken up by the lectin receptor may be processed and presented normally.

There was no obvious difference in the phenotypes of the peritoneal cell populations obtained from normal and alloxan treated mice (Table 3). In addition, the adherent cell population derived from diabetic and normal mice had similar profiles. This indicates that the observed decrease in proliferation was not due to the loss of an important subset of cells after alloxan treatment. Our earlier observation that there is an association between the lectin receptor binding *Staph. epidermidis* and Ia antigen of the MHC (Stewart, Glass & Weir, 1982) suggests that the function of the lectin receptor may be involved in presentation of antigen in the context of class II molecules to MHC restricted T helper cells. Thus reduction in lectin receptor expression in diabetes may alter Ia function. In the present report, however, we were unable to demonstrate any concomitant reduction in Ia expression. However the antibody used (A.TH anti A.TL) has a broad specificity for all the I region gene products and it is possible that a specific alteration in one of the alpha or beta chains of I-A or I-E may not have been detected under the present assay conditions. It should also be noted that the assay for Ia antigen is not a functional assay and only detects the presence of the I region gene product on the surface of the macrophages.

Our results so far indicate that the diabetic environment affects the macrophage plasma membrane thereby influencing macrophage recognition and function. Down-regulation of the lectin receptor in diabetes profoundly alters the initial stages of the immune response. Presentation of bacterial antigens is defective and this has important implications for the ability of the diabetic individual to mount an effective immune response.

REFERENCES

- AHMANN, G.B., SACHS, D.S. & HODES R.J. (1978) Requirement for an Ia-bearing accessory cell in Con A-induced T cell proliferation. *J. Immunol.* **121**, 1981.
- EDWARDS, M.S. & FUSELIER P.A. (1983) Enhanced susceptibility of mice with streptozotocin-induced diabetes to Type II Group B Streptococcal infection. *Infect. Immun.* **39**, 580.
- EZEKOWITZ, R.A.B., AUSTYN, J., STAHL, P.D. & GORDON S. (1981) Surface properties of bacillus calmette-guerin-activated mouse macrophages. Reduced expression of mannose-specific endocytosis, Fc receptors and antigen F4/80 accompanies induction of Ia. *J. exp. Med.* **154**, 60.
- FLETCHER-MCGRUDER, B.L. & GERRITSEN, G.C. (1984) Deficient humoral antibody response of the spontaneously diabetic Chinese hamster (41769). *Proc. Soc. exp. Biol. Med.* **175**, 74.
- FREIMER, N.B., OGMUNDSOTTIR, H.M., BLACKWELL, C.C., SUTHERLAND, I.W., GRAEME, L. & WEIR, D.M. (1978) The role of cell wall carbohydrates in binding of microorganisms to mouse peritoneal exudate macrophages. *Acta Path. Microbiol. Scand. Sect. B.* **86**, 53.
- GANDA, O.P. (1983) Morbidity and mortality from Diabetes Mellitus. A look at preventable aspects. *Am. J. Public Health.* **73**, 1156.
- GLASS, E.J., STEWART, J. & WEIR, D.M. (1981) Presence of bacterial binding 'lectin-like' receptors on phagocytes. *Immunol.* **44**, 529.
- GLASS, E.J., STEWART, J. & WEIR, D.M. (1982) The influence of chemotactic factors on the expression of phagocyte receptors. *J. Lab. clin. Immunol.* **7**, 39.
- GLASS, E.J., STEWART, J. & WEIR, D.M. (1983) Membrane changes in murine macrophages after *in vitro* stimulation and activation. *Immunol.* **50**, 165.
- IMBER, M.J., PIZZO, S.V., JOHNSON, W.J. & ADAMS D.O. (1982) Selective diminution of the binding of mannose by murine macrophages in the late stages of activation. *J. biol. Chem.* **257**, 5129.
- JULIUS, M.F., SIMPSON, E. & HERZENBERG, L.A. (1973) A rapid method for the isolation of functional thymus-derived murine lymphocytes. *Eur. J. Immunol.* **3**, 645.
- PASKO, K.L., SALVIN, S.V. & WINKLESTEIN, A. (1981) Mechanisms in the *in vivo* release of lymphokines. V. Responses in alloxan-treated and genetically diabetic mice. *Cell Immunol.* **62**, 205.
- PIZZO, S.V., LEHRMAN, M.A., IMBER, M.J. & GUTHROW, C.E. (1981) The clearance of glycoproteins in diabetic mice. *Biochem. Biophys. Res. Comm.* **101**, 704.
- PRUD'HOMME, G.J., FUKS, A., COLLE, E., SEEMAYER, T.A. & GUTTMAN, R.D. (1984) Immune dysfunction in diabetes-prone BB rats. Interleukin-2 production and other mitogen induced responses are suppressed by activated macrophages. *J. exp. Med.* **159**, 463.
- STEWART, J., GLASS, E.J. & WEIR, D.M. (1982) Macrophage binding of *Staphylococcus albus* is blocked by anti-I-region alloantibody. *Nature (Lond.)* **298**, 852.
- SUMMERFIELD, J.A., VERGALLA, J. & JONES E.A. (1982) Modulation of a glycoprotein recognition system on rat hepatic endothelial cells by glucose and diabetes mellitus. *J. Clin. Invest.* **69**, 1337.
- WEIEL, J.E. & PIZZO, S.V. (1983) Down regulation of macrophage mannose/N-acetylglucosamine receptors by elevated glucose concentrations. *Biochem. Biophys. Acta*, **159**, 170.
- WEIR, D.M., BLACKWELL, C.C. & MCLEAN, C.A. (1981) Impaired bacterial binding to peritoneal exudate cells from mice with alloxan induced diabetes. *J. lab. clin. Immunol.* **5**, 37.